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Discovery of bis-aromatic ring neonicotinoid analogues fixed as *cis*-configuration: Synthesis, insecticidal activities, and molecular docking studies

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ABSTRACT

A novel series of bis-aromatic ring neonicotinoid analogues (**1a–1l**, **2a–2c**), were designed and prepared by introducing a new substituted aromatic ring into nitenpyram and forming a tetrahydropyrimidine ring, with the *cis*-configuration confirmed by X-ray diffraction. Preliminary bioassays showed most analogues exhibited good insecticidal activities at 100 mg/L, and compound **1d** and **2a** were highly potent even at 10 mg/L. Modeling the ligand–receptor complexes by molecular docking study explained the structure–activity relationships observed in vitro, which may provide some useful information for future design of new insecticides.

The discovery of neonicotinoid insecticides (NNs) can be considered as a milestone in agrochemical research over the past three decades. NNs have recently received global interests from both agricultural chemistry and medicinal fields, due to their broad spectrum of biological activities and high selectivity for public health applications[1] and crop protection. In 2006, the neonicotinoid family accounted for worldwide annual sales of around $US 1.56 billion, representing nearly 17% of the global insecticide market.[2] Neonicotinoids represented by imidacloprid[3] act on the insect nicotinic acetylcholine receptors (nAChRs), with a much lower affinity for the mammalian nAChR,[4,5] which makes this class of insecticides particularly attractive to the primarily controlled sap-feeding pests[6–8] and relatively safe toward mammals.[9,10]

However, during the past decades, frequent field applications have inevitably led to insects resistance to the major class of NNs.[11–13] Significant increases in cross-resistance have also been observed in a range of species,[14–18] with more recently collected strains exhibiting more than 100-fold resistance to imidacloprid and comparable levels of resistance to thiamethoxam and acetamiprid.[19,20] Therefore, the development of new potent neonicotinoids with novel chemical structures and low resistance is of an urgent desire.

Until recently, most of the researches about structure optimization of NNs are based on cyclic neonicotinoid insecticides, such as imidacloprid. However, few studies have been focused on the structural modification of acyclic NNS, such as nitenpyram and acctamiprid. Encouraged by this, we developed a new structure developing strategy, using nitenpyram as the lead compound (Fig. 1), and a series of novel substituted-1,2,3,6-tetrahydropyrimidine derivatives were designed by introducing a new substituted aromatic ring into nitenpyram and forming a tetrahydropyrimidine ring fixed as *cis*-configuration. As a result, most of these analogues exhibited significant insecticidal activities against *Nilaparvata legen*, and some of them had >90% mortality at 10 mg/L. This paper describes the syntheses, bioactivities and the structure–activity relationship. The interactions between these new compounds with

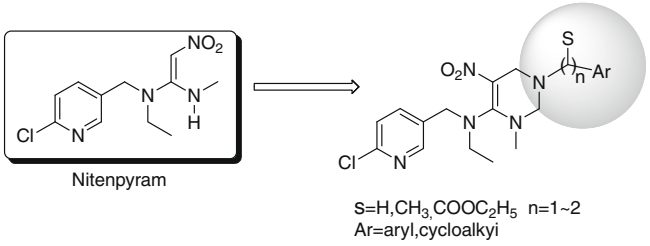

Figure 1. Chemical structures of the target compounds.

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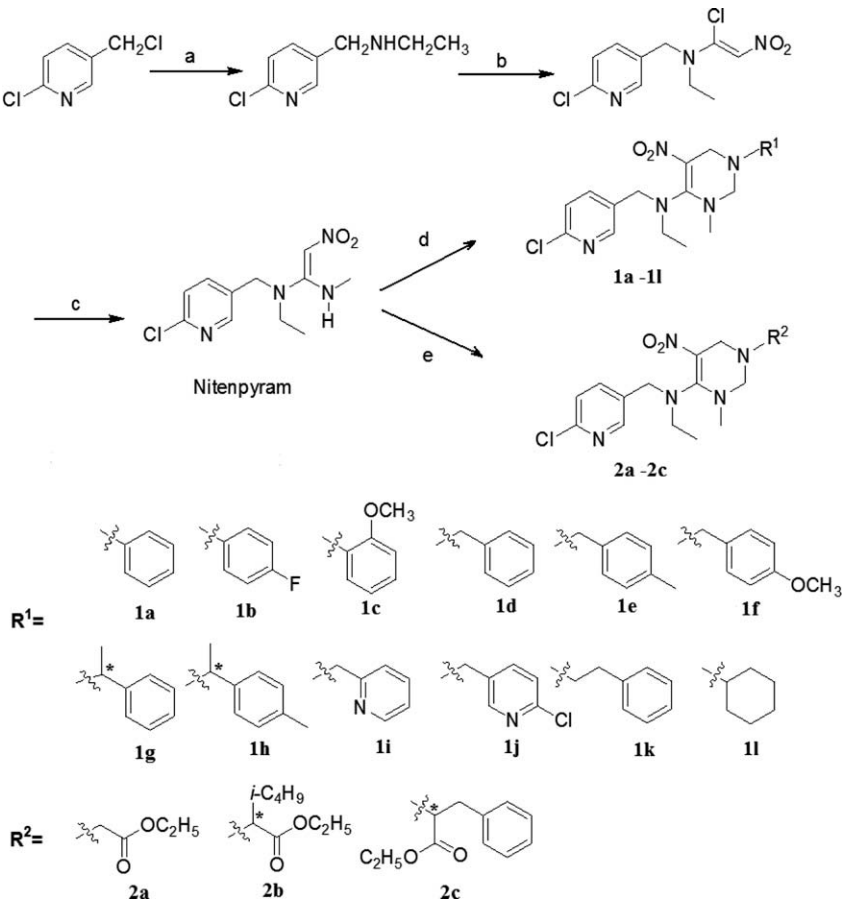


Scheme 1. Preparation of substituted 1,2,3,6-tetrahydropyrimidines (**1a**–**1l**, **2a**–**2c**). Reagents and conditions: (a) ethanamine (42%); (b) 1,1,1-trichloro-2-nitroethane/$CHCl_3$ 2–7 °C (65%); (c) methanamine 3–7 °C (58%); (d) various substituted amine, 37% HCHO, EtOH, 25 °C (65–80%); (e) L-α-amino acid ethyl ester hydrochloride, 37% HCHO, Et_3N/EtOH, 70 °C, refluxing (71–78%).

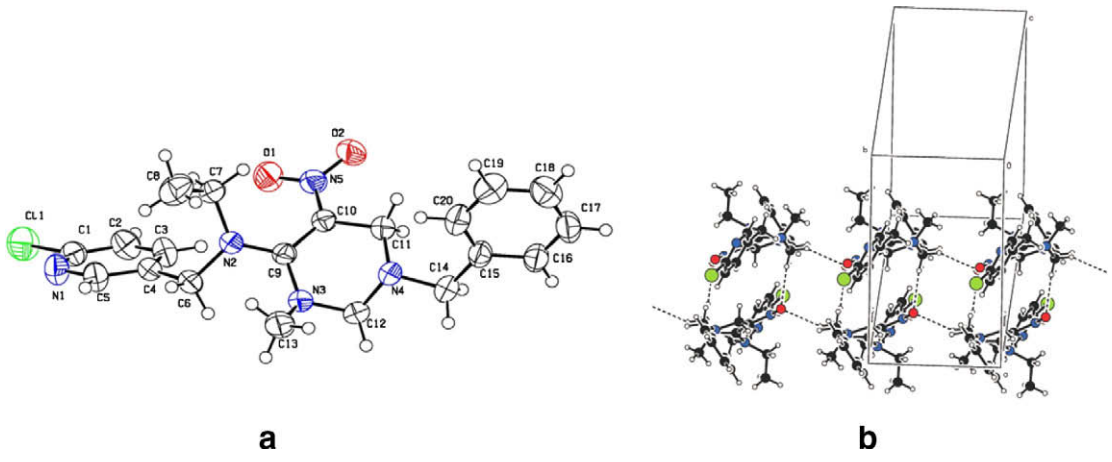
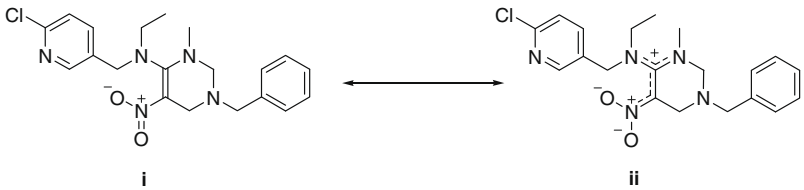


Figure 2. Molecular structure of compound **1d** (CCDC number 746864) with atom-labeling and the packing diagram.



Scheme 2. Resonance of the structures **i**/**ii**.

their target nAChRs were also investigated by molecular docking, which may provide some useful information for future design of new insecticides.

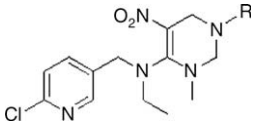
Synthesis procedures for these new substituted-1,2,3,6-tetrahydropyrimidine neonicotinoid analogues are summarized in Scheme 1. Nitenpyram, which was prepared based on the procedures previously reported,[21] was reacted with various substituted amine and formaldehyde in acetate acid, affording desired compounds **1a**–**1l** with *cis*-configuration fixed by 1,2,3,6-tetrahydro-pyrimidine. Meanwhile, compounds **2a**–**2c** with *cis*-configuration were also obtained by Mannich reaction. The mixture of Nitenpyram, L-α-amino acid ethyl ester hydrochlorides and formaldehyde was stirred for 30 min in a microwave reactor, which may speed up reactions with good yields. The structures of the target compounds were well characterized by ^{1}H NMR, MS, EA, IR, and elemental analysis.

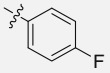
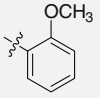
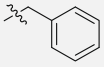
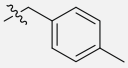
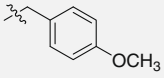
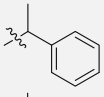
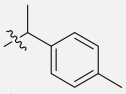
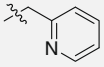
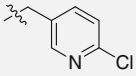
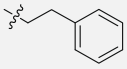
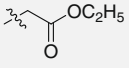
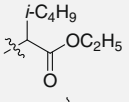
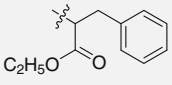
To further obtain precise three-dimensional structural information, compound **1d** was recrystallized by slow evaporation from mixed solution of compound **1d** in ethyl acetate/petroleum ether (v:v = 1:4), and its single-crystal structure was confirmed by X-ray crystallographic data analysis (Fig. 2).[22] The crystal data have been listed in the Supplementary data associated with this Letter. Due to the lone-pair electrons transferring on the amines to C(9)=C(10), the C9–N2 and C9–N3 bond lengths, both 1.36 Å, are remarkably shorter than typical C–N single bond (1.47 Å) but close to C=N (imine) (1.33 Å).[23] A coplanar olefin–amine π-electron network (Scheme 2) is formed, owing to the delocalization of the electrons which extend to the strong electron-withdrawing group, $-NO_2$. In addition, the bond lengths of C9=C10 and C10–N5 are 1.40 and 1.39 Å, which are longer than that of pure C=C (1.34 Å) and shorter than that of typical C–N in $C-NO_2$ (1.49 Å). In the symmetric unit, an intramolecular hydrogen bond (C3–H3···O1) forming a nine-membered ring (O1, N5, C10, C9, N2, C6, C4, C3, H3) was observed, and two intermolecular hydrogen-bonds are found between molecules (Fig. 2b), forming a three-dimensional interaction network, which may help stabilize the crystal structure. Besides, The most remarkable feature of this molecule is the *cis*-configuration of its nitro group, and the preference for this geometry is mostly due to the newly introduced tetrahydropyridine ring that fixes the nitro moiety in the *cis* position. Since most of the crystal structures of neonicotinoid insecticides were determined with *trans*-configuration, which all the studies of mechanism were based on, this new unique *cis*-configuration may endue these new compounds with a novel mode of action.

The insecticidal activities of these novel analogues were evaluated against *Nilaparvata legen*.[24] As depicted in Table 1, most compounds exhibited good in vitro insecticidal activities against *Nilaparvata legen* and had >80% mortality at 100 mg/L. Among these compounds, **1d** and **2a** showed significant potency that is close to nitenpyram at 10 mg/L, and the bioactivities of (**1b**, **1g**, **1j**, and **1k**) were slightly weaker than that of nitenpyram. Compared with **1a**, the introduction of cycle alkyl (**1l**) in stead of aromatic groups showed decreasing tendency in insecticidal activity. Interestingly, the introduction of F and Cl elements into the aromatic groups resulted in increased insecticidal activity (**1a** vs **1b**, **1i** vs **1j**), and compounds containing phenyl displayed higher inhibitory activity than the corresponding derivatives with heterocycle such as pyridinyl (**1d** vs **1i**).

For the effects of the flexible linkage at 1-position of tetrahydropyrimidine ring, the insecticidal activities of the corresponding analogues decreased in the order benzyl (**1d**) > phenylethyl (**1k**) > phenyl (**1a**), and the introduction of a large ester group into the linkage resulted in remarkably decreased insecticidal activity (**2c** vs **1k**). These observations clearly suggested that the length and flexibility of the linkage was strongly related to their insecticidal potency.

Table 1
Insecticidal activities of the target compounds (**1a**–**1l**, **2a**–**2c**) against *Nilaparvata legen*



Compd	R	Concn.[a] (mg/L)		
		500	100	10
1a		++++++	+++++	+++
1b		++++++	++++++	++++
1c		++++++	++++++	++
1d		++++++	++++++	+++++
1e		++++++	+++	+
1f		+++++	+	n.t.[b]
1g[c]		++++++	++++++	++++
1h[d]		+++++	++++	+++
1i		++++++	++++	+++
1j		++++++	++++++	++++
1k		++++++	++++++	++++
1l		+++++	++++	++
2a		++++++	++++++	+++++
2b[d]		+++	+	–
2c[d]		++++	+++	++
Nitenpyram[b]		++++++	++++++	++++++

[a] Rating system for the mortality percentage: ++++++, 100%; +++++, ≥90%; ++++, ≥80%; +++, ≥70%; ++, ≥60%; +, ≥50%; –, <50%.
[b] n.t. = not tested.
[c] (+)-.
[d] (±)-.

To further explore the structural features for better activities, models of these new compounds–receptor complexes were investigated by docking studies with AutoDock version 4.0.[25] Since the amino acids forming the active sites are both structurally and functionally consistent in the diverse nAChRs and AchBPs, the published crystal structure of a *Lymnaea stagnalis*-AChBP (*Ls*-AChBP) co-crystallized with imidacloprid (PDB ID: 2zju)[26] was used as

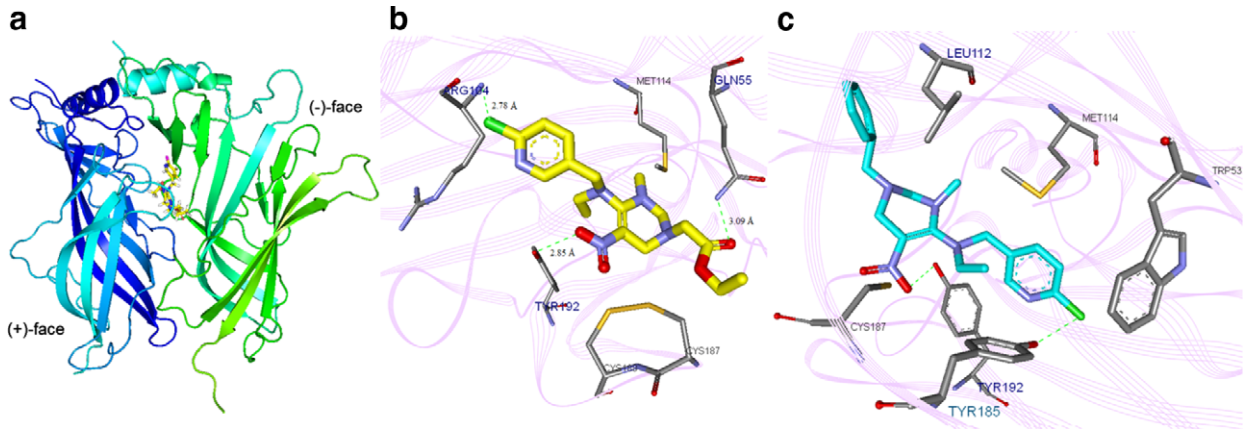


Figure 3. Modeling of the docking results of compounds **1d**, **2a** in the extracellular domain of nAChR. (a) **2a** nestled in the interfacial agonist-binding pocket between the (+)-face (primary, yellow) and (−)-face (complementary, blue) subunits of the *Ls*-AChBP (PDB ID: 2zju), as a structural surrogate of the insect nAChR; (b) nAChR-**2a** binding site interactions (zoomed-in) featuring hydrogen-bonding between **2a** and the active site residues; (c) nAChR-**1d** interactions. The second structure of the protein is represented as line ribbon.

the template of receptor. The docking was carried out through the graphical user interface AUTODOCKTOOLS (ADT 1.4.6). The only modification was the number of docking runs that was set to 200 (previously 100) for more accuracy.

As a result, the scoring function of the docking program ranked the compounds in the same general order observed experimentally (data not shown), and all active analogues exhibited significant hydrogen-bonding interactions with the nAChR target. As expected, the most potent compound **2a** is nicely accommodated within the subunit interfacial binding pocket between the two faces of adjacent subunits (Fig. 3a). Its binding conformation exhibited important hydrogen bond between its nitro O21 and H–O of Tyr192 (O–H···N: 2.85 Å, 138.6°) (Fig. 3b), and the O24 of its ester group hydrogen-bonds the side chain O of Gln55, while the chloropyridine interacts primarily with Arg104. Other interactions in the active site region may be mediated via water(s), since these residues are near the surface of the receptor. In addition, analogue **1d** binds to the nAChR with comparable affinity to **2a** (Fig. 3c), which is consistent with its high insecticidal activity. Besides, important additional hydrophobic interactions have been found between the side chain of Leu112 and its phenyl, which is newly introduced to these bis-aromatic ring neonicotinoid analogues. These observations have also explained the structure–activity relationships observed in vitro.

Furthermore, most of the other active derivatives (**1b**, **1g**, **1j**, **1k**) shared a quite similar binding mode with **2a**, and many of them exhibited more than two hydrogen-bonds with different amino acids of the active pocket between the nAChR subunits (unpublished result). These amino acids (Gln73, Trp 143, Cys187, Cys188, and Glu190) were different from the ones that interacted with imidacloprid, which suggested a novel mechanism of the insecticidal effect by these new compounds. Thereby, the newly introduced substituents of the designed analogues presumably played important roles in ligand recognition and binding interactions, which may further enhance their activities and contribute to the selectivity as well. Based on these, further target inhibitory tests and advanced insecticide design are underway.

In summary, a series of novel bis-aromatic ring neonicotinoid analogues, which were designed based on the acyclic NNS (nitenpyram), were synthesized and tested for their insecticidal activity against *Nilaparvata legen*. All the target compounds presented good insecticidal activity at 100 mg/L. Among these analogues, **1d** and **2a** afforded the best in vitro activity, and had >90% mortality at 10 mg/L. The single-crystal structure of **1d** was further determined by X-ray diffraction, with the *cis*-configuration of its C9═C10 confirmed. In addition, molecular docking studies were also carried out to model the ligand-nAChR complexes and analyze their interactions for improved activity. The docking results revealed a unique binding mode other than nitenpyram, and were in good agreement with their high insecticidal potential, which also explained the structure–activity relationships observed in vitro. Further researches are underway to verify the nAChR target and evaluate their inhibitory activities against resistant insect species. The study herein has shed a light on the mechanism of action of these bis-aromatic ring neonicotinoid analogues, thereby prompting some useful information for future design of new NNs pesticides.

Acknowledgments

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Supplementary data

Supplementary data (experimental details include full synthesis procedures, analytical characterization of all the compounds and the crystallographic data of compound **1d**) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.050.

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